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Research Paper

Activation of dsRNA Dependent Protein Kinase PKR in Karpas299 Does Not Lead to Cell Death

Inbar Friedrich[1]
Hannah Ben-Bassat[2]
Alexander Levitzki[1,*]

[1]Unit of Cellular Signaling; Department of Biological Chemistry; The Alexander Silberman Institute of Life Sciences; The Hebrew University of Jerusalem; Givat Ram, Jerusalem Israel

[2]The Laboratory of Experimental Surgery; Hadassah University Hospital; Jerusalem Israel

*Correspondence to:Alexander Levitzki; Unit of Cellular Signaling; Department of Biological Chemistry; The Alexander Silberman Institute of Life Sciences; The Hebrew University of Jerusalem; Givat Ram, Jerusalem 91904 Israel; Tel.: 972.2.658.5404; Fax: 972.2.651.2958; Email: levitzki@vms.huji.ac.il

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KEY WORDS

PKR, p67, T-cell non-Hodgkin's lymphoma, NPM/ALK, RNAi, eIF2α, translocation

ABBREVIATIONS

AS	antisense
dsRNA	double-strandedRNA
EGFR	epidermal growth factor receptor
NHL	non-Hodgkin's lymphoma
PolyIC	poly inosine-cytosine
RNAi	RNA interference

ACKNOWLEDGEMENTS

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ABSTRACT

Activated double-stranded RNA (dsRNA)-dependent protein kinase PKR is a potent growth inhibitory protein that is primarily activated in virally infected cells, inducing them to die. We have recently shown that PKR can be selectively activated in cancer cells, by in situ generation of dsRNA following introduction of antisense RNA complementary to an RNA expressed specifically in the cancer cell. The feasibility of this approach was demonstrated using a glioblastoma line that overexpresses a truncated form of the EGFR. PKR and its signaling pathway are not restricted to a given cell line; therefore, in principle, this dsRNA killing approach can be applied to any cancer that expresses unique RNA sequences. Nonetheless, applying this approach to Karpas299 cells, from a T-cell non-Hodgkin's lymphoma that harbors the NPM/ALK translocation, did not result in cell death, implying that PKR signaling pathway is repressed in this cell line. Indeed, the phosphorylation of eIF2α by PKR was impaired in Karpas299 cells. Furthermore, levels of the cellular inhibitor p67 were elevated in these cells. Long antisense, as well as RNAi for p67, delivered into Karpas299 cells by adenoviruses, reduced p67 levels. The reduction in p67 levels led to increased phosphorylation of eIF2α, and an additive effect was achieved by coinfection with NPM/ALK-AS encoding adenoviruses. Infection with these adenoviruses, however, did not promote growth inhibition. These findings imply that anti-apoptotic mechanisms counteract PKR signaling in this T-cell nonHodgkin's lymphoma.

INTRODUCTION

The double stranded (ds)RNA-dependent protein kinase, PKR, is a potent growth inhibitory protein that is most frequently activated in virally infected cells, inducing them to die. PKR is a serine/threonine protein kinase that phosphorylates the α-subunit of the protein synthesis initiation factor eIF2, resulting in sequestration of the GDP-GTP exchange factor eIF2B and inhibition of translation.[1] Activation of PKR involves two molecules binding in tandem to dsRNA and subsequently phosphorylating each other.[2] dsRNA molecules shorter than 30 base pairs (bp) fail to bind PKR and do not activate the enzyme. Molecules longer than 30 bp bind and activate the enzyme with an efficiency that increases with increasing chain length, reaching a maximum at ~85 bp.[3]

In many cancers, chromosomal rearrangements and truncations lead to the production of unique mRNA species. In principle, a cancer cell can be infected with a viral vector encoding 30- to 85-nucleotide long antisense (AS) RNA, which complements the unique mRNA sequence flanking the fusion point or truncation. Upon hybridization, a dsRNA molecule will be generated that is sufficiently long to activate PKR (Fig. 1). In non-cancerous cells, however, the flanking sequences will be far apart, and only short dsRNA species will be formed. These short dsRNA species will not be long enough to activate PKR. Thus, if the AS RNA is expressed in all cells, only the cancer cells will die. The hypothesis that PKR activation can be harnessed for selective killing of cancer cells was successfully validated in our laboratory by utilizing a glioblastoma cell line that expresses a truncated form of epidermal growth factor receptor (EGFR) mRNA.[4] PKR and its signaling pathway are not restricted to a given cell line, therefore, in principle, this dsRNA killing approach can be applied to any cancer that expresses unique mRNA sequences.

Harnessing PKR for the selective killing of cancer cells, could serve as a powerful strategy for treating cancer. We have shown that activation of PKR can lead to cell death in glioblastoma, both in vitro and in vivo.[4] In the current work, we have attempted to apply the dsRNA killing approach to selectively kill a T-cell nonHodgkin's lymphoma (NHL) that expresses the NPM/ALK fusion, using the Karpas299 cell line[5] as a model for this lymphoma. Introduction of 47-nucleotide long antisense complimentary to the NPM/ALK

fusion did not kill the cells. This result suggested that PKR activity or its signaling pathway is impaired or inhibited in this cell line. In this study we investigate mechanisms that can interfere with the PKR induced cell death.

MATERIALS AND METHODS

Materials

All cell culture growth media and sera were purchased from Biological Industries, Beit Haemek, Israel. PolyIC was from Sigma, USA, tunicamycin from Calbiochem, USA, and sodium arsenate from J.T Baker Chemicals, USA. The following antibodies were used in this research: anti-PKR antibody (RiboGene, USA), antibody against the phosphorylated form of eIF-2α (Research Genetics, USA), anti-eIF-2α, anti-actin, anti-GFP were all purchased from Santa Cruz, USA, and anti-p67, which was a generous gift from Prof. B. Datta.[6] Anti-goat, anti-rabbit and anti-mouse antisera conjugated with HRP were obtained from Jackson Immuno-Research Laboratories, Inc (Town, USA).

Plasmids

Plasmid pcDNA-3-NPM/ALK was a gift from Prof. J. Duyster (Technical University of Munich, Germany).

Plasmid pAdeTrack-U6-NPM/ALK-AS. The 47-nt long antisense oligonucleotide corresponding to the NPM/ALK fusion point was synthesized and cloned between the *Xho*I and *Nsi*I sites of the U6 expression plasmid pGEM-2 obtained from Dr. J.R Grandis.[7] The antisense sequence is as follows:TCGAGGTGCTTCCGGCGGTACAC-TACTAAGTGCTGTCCACTAATGCA. For adenoviral vector production the U6 cassette was excised from plasmid pGEM-2-NPM/ALK-AS by *BamH*I/*EcoR*I digestion, "filled in" and ligated into the *EcoR*V site of adenovector pAdeTrack,[8] to generate the plasmid pAdeTrack-U6-NPM/ALK-AS.

Plasmid pAdeTrack-CMV-p67-AS. A 1.5 kb RT-PCR product (see below), representing p67 mRNA from Karpas299 cells, was sub cloned into vector pDrive (QIAGEN, Germany). The resulting plasmid was cut with *Kpn*I/*Hind*III and the fragment encoding p67 was cloned in its antisense orientation into adenoviral vector pAdeTrack-CMV[8] cut with the same enzymes, to generate the plasmid pAdeTrack-CMV-p67-AS.

Plasmid pAdeTrack-U6-p67siRNA. Four putative siRNA sequences were chosen using the "siRNA target finder and design tool" (www.ambion.com). These sequences were synthesized and cloned into the *Xho*I and *Nsi*I sites of the U6 expression plasmid pGEM-2 obtained from Dr. J.R Grandis.[7] The ability of these siRNA sequences to reduce p67 levels was first examined in HEK293 cells, which are easily transfected. siRNA#17 and siRNA #105 sequence, which affected p67 levels in HEK293 cells (data not shown), were sub-cloned into the adenoviral vector as follows. Plasmids pGEM-2-U6-p67siRNA#17 and #105 were cut with *EcoR*I/*BamH*I, "filled in" and cloned into the *EcoR*V site of vector pAdeTrack,[8] to generate plasmid pAdeTrack-U6-p67siRNA#17 and #105.

RT-PCR

RNA was produced from Karpas299 cells using Tri reagent (Sigma, USA), according to the manufacturer's instructions. RT-PCR was carried out using the One Step RT-PCR kit (QIAGEN, Germany), according to the manufacturer's instructions. For amplification of p67 mRNA we used the following primers: hp67-5': 5' CGCGCTCTCTCGGGCAAC 3' and hp67-3': 5' AAGGTGTTGAGGTG GCTTTGG 3'. For amplification of eIF2α mRNA we used the following primers: eIF2α-5': 5' CCTGAGGTGGAAGATG-TAGTG '3 and eIF2α-3': '5 ctgtagaac aattcaaacctgc '3. For amplification of

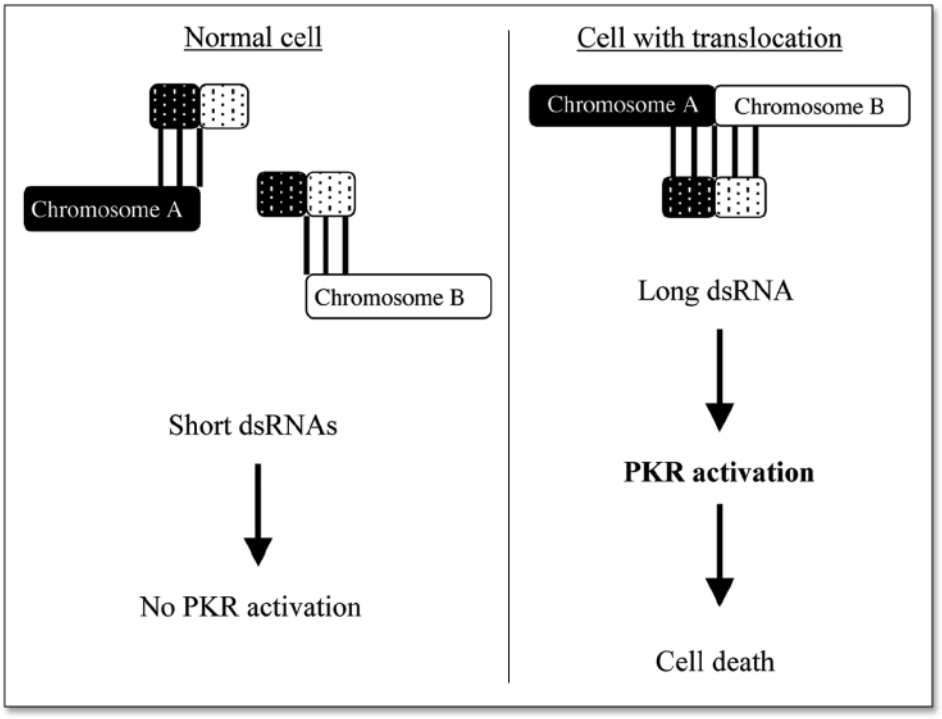

Figure 1. Mechanism for specific activation of PKR in cells harboring a translocation = Oligo antisense RNA.

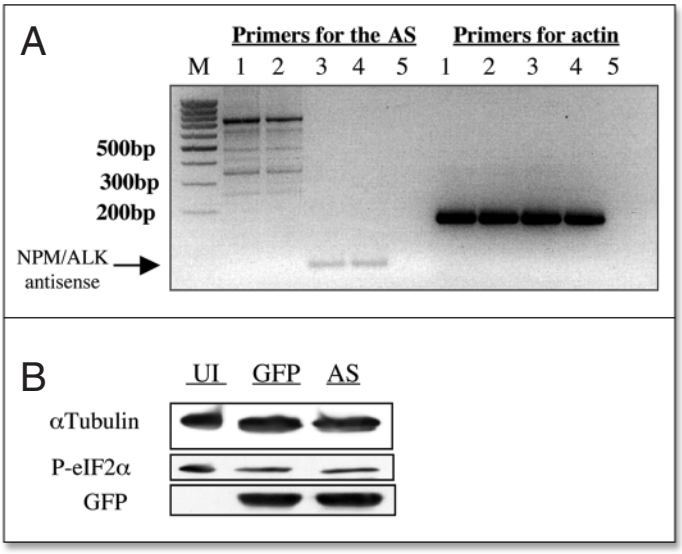

Figure 2. Expression of NPM/ALK AS in Karpas299 cells does not lead to eIF2α phosphorylation. Karaps299 cells were infected with adenoviruses encoding NPM/ALK antisense or GFP. (A) Antisense expression was determined by RT-PCR, as described in Materials and Methods. Expression of NPM/ALK antisense (47 bp) was seen only in cells that were infected with adenoviruses encoding NPM/ALK antisense (lanes 3 and 4) and not in uninfected cells (lane 1) or in cells infected with adenoviruses encoding GFP (lane 2). Lane 5 contained the reaction mixture without RNA. An Actin RT-PCR product was obtained from all samples, indicating the presence and quality of the RNA produced from the various samples. (B) eIF2α phosphorylation was not induced in cells infected with adenoviruses encoding the NPM/ALK antisense. UI, uninfected; GFP, infected with adenoviruses encoding GFP; AS, infected with adenoviruses encoding NPM/ALK antisense.

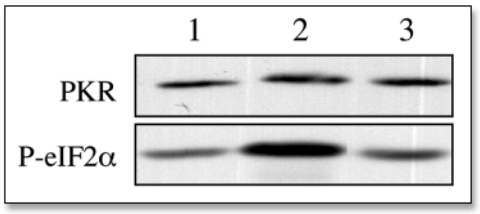

Figure 3. PKR activation in HEK293 cells by cotransfection of NPM/ALK and NPM/ALK-AS encoding plasmids. The ability of the NPMALK-AS to bind NPM/ALK and activate PKR was tested as follows: HEK293 cells were transfected with NPM/ALK-AS encoding plasmid alone (lane 3), or together with NPM/ALK encoding plasmid (lane 2). 48 hours after transfection, the cells were lysed and a cell-free nonradioactive PKR kinase assay was preformed as described in Materials and Methods. At the end of the reaction samples were electrophoresed and blotted. The blot was then probed with anti-PKR and anti-P-eIF2α antibodies. As can be seen, phosphorylation of the recombinant substrate was obtained only in lysates derived from cells that were transfected with both plasmids (lane 2) as opposed to nontransfected cells (lane 1) or to cells transfected with NPM/ALK-AS encoding plasmid alone.

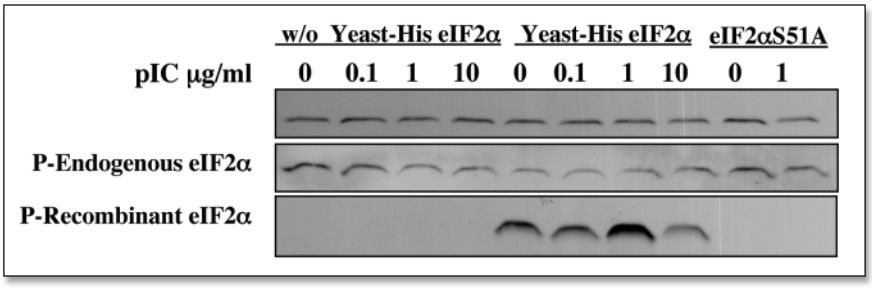

Figure 4. PKR activity in whole Karpas299 whole cells lysates. Cell-free nonradioactive PKR kinase assay was performed as described in "Materials and Methods". At the end of the reaction samples were electrophoresed and blotted. The blot was then probed with anti-PKR and anti-P-eIF2α antibodies. PKR was activated upon addition of pIC, as can be seen by the change in the phosphorylation state of the recombinant substrate Yeast-His-eIF2α. No change was observed in the phosphorylation state of the endogenous eIF2α upon addition of pIC.

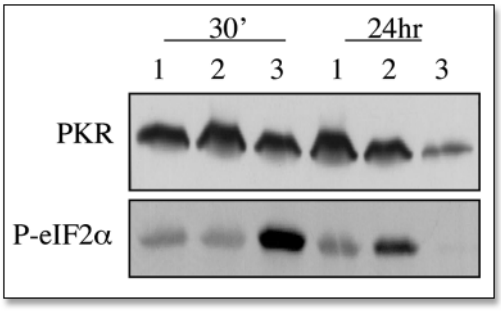

Figure 5. Treatment of Karpas299 cells with stress-inducing agents induced eIF2α phosphorylation. Karpas299 cells were treated with: 1. DMSO 2. Tunicamycin (25 μg/ml) or 3. Sodium arsenate (100μM), and harvested 30 minutes or 24 hours after treatment. The blot was probed with anti-PKR and anti-P-eIF2α antibodies. Treatment with arsenate and tunicamycin led to a dramatic elevation in eIF2α phosphorylation, 30 minutes and 24 hours after treatment, respectively.

actin we used the following primers: Actin 822(+): '5 TGAAACAACARACAATTCCATCATGAAGTGTGAC 3' and Actin 966(-): '5 AGGAGCGATAATCTTGATCTTCATGGTGCT3'. Two-step RT-PCR for amplification of NPM/ALK antisense: RT reaction was done with primer RT-pGEM-2: '5 TATGGAACGCTTCATGCA 3', followed by PCR reaction with both primers RT-pGEM-2 and NPM AS: '5 CGTGCTCGAGGTGCTTCC 3'.

Cell culture

Karpas299 cells were a gift from Prof. J. Duyster (Technical University of Munich, Germany). The cells were cultured in suspension in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS) and streptomycin (100 μg/L) and penicillin (100,000 units/L). The HEK293 cell line was grown in DMEM supplemented with 10% FCS and antibiotics. The glioblastoma cell line U87MG-wtEGFR, which overexpresses the EGFR, was grown in DMEM supplemented with 10% FCS and antibiotics, in the presence of 400 μg/ml G418.

Adenovirus production

Adenoviruses were produced as described.[8] In brief, recombinant adenoviral plasmids were generated by homologous recombination between the various transfer vectors (pAdeTrack or pAdeTrack-CMV) and the plasmid pAdeEasy-1 in *E. coli* BJ5183. HEK293 cells, grown in DMEM supplemented with 10% FCS and antibiotics, were transfected with the recombinant adenoviral plasmids and virus enrichment cycles were performed. Adenoviruses were easily detected by fluorescence microscopy during the enrichment cycles, as the vector coded for the green fluorescence protein (GFP) in addition to NPM/ALK antisense, p67 antisense and p67 siRNAs.

Infection by spinoculation

Infection by spinoculation was performed as described[9] with some changes. 1.5 ml microfuge tubes, containing the desired number of cells in 10 μl growth medium (supplemented with 2% FCS) and 10 to 20 μl viruses, were centrifuged at room temperature for 90 minutes at 1200 x g. At the end of the spinoculation, the cells were transferred to tissue culture plates containing fresh medium supplemented with 10% FCS and antibiotics.

Cell-free non-radioactive PKR kinase assay

Cells were washed in cold PBS and lysed with Lysis Buffer (20 mM Tris-HCl pH 7.5, 5 mM $MgCl_2$, 50 mM KCl, 400 mM NaCl, 2mM DTT, 20% Glycerol, 10 μg/ml AEBSF, 10 μg/ml Aprotinin, 1% Triton X-100). Thereafter, the samples were centrifuged at 10,000 x g for 10 min at 4°C to remove cell debris. Fifty micrograms of each sample were taken for the kinase reaction. The reaction was carried out in total volume of 100 μl in Reaction Buffer (20 mM Tris-HCl pH 7.5, 5 mM $MgCl_2$, 50 mM KCl, 2 mM $MnCl_2$, 5% Glycerol, 10mM NaF, 100 μM ATP, 10 μg/ml AEBSF 10 μg/ml Aprotinin). PKR was activated by adding PolyIC to various final concentrations (0.1, 1 or 10 μg/ml) followed by incubating the samples for 10 minutes on ice. In some cases, as will be indicated, 0.5 μg of the recombinant substrate yeast-His-eIF2α-wt or yeast-His-eIF2αS51A[10] were added to the samples. The reaction was started by incubating the samples at 30°C, and stopped after 20 minutes by adding Laemmli Buffer[11] and boiling at 100°C for 10 minutes. Samples were then electrophoresed and blotted. The blots were probed with various antibodies, as indicated in the Results, using the ECL procedure. When using HEK293 lysates, the reaction was performed in the presence of the recombinant substrate yeast-His-eIF2α-wt, without addition of PolyIC.

Determination of eIF2α phosphorylation in Karpas299 cells

Karpas299 cells were infected with adenoviruses encoding NPM/ALK antisense. 48hr after infection the cells were lysed. 50 μg of each sample were electrophoresed and blotted. The blots were probed with the antibodies anti-αTubulin (Santa Cruz Biotechnology, USA; 1:20,000), anti-P-eIF2α (Research Genetics, USA; 1:10,000) and anti-GFP (Santa Cruz Biotechnology, USA; 1:10,000) and visualized using the ECL procedure.

RESULTS AND DISCUSSION

Infection of Karpas299 cells with NPM/ALK as did not result in cell death. We attempted to kill Karpas299 cells by infecting them with an adenovirus vector encoding antisense complimentary to the NPM/ALK transcript. Although the antisense RNA was transcribed (Fig. 2A), the cells did not die (data not shown). Moreover, we did not detect phosphorylation of eIF2α following antisense expression in these cells (Fig. 2B). The same antisense vector did induce phosphorylation of recombinant eIF2α in lysates derived from HEK293 cells that had been transduced with a vector encoding the NPM/ALK fusion protein (Fig. 3).

These findings imply that the PKR signaling pathway is impaired or repressed in the Karpas299 cell line. We therefore examined whether eIF2α can be phosphorylated at all in this NHL T-cell line.

No change in eIF2α phosphorylation upon in vitro activation of PKR by dsRNA. We examined dsRNA-induced PKR activation in a cell-free system, because Karpas299 were refractory to transfection. We lysed Karpas299 cells and measured PKR activity in whole lysates by a cell-free nonradioactive PKR kinase assay, as described in "Materials and Methods". PolyIC was added to four samples of the lysate, to 0, 0.1, 1 or 10 μg/ml. The recombinant substrate yeast-His-eIF2α-wt was added to each of these samples. As negative controls, the recombinant substrate yeast-His-eIF2α-S51A, a mutant form which cannot be phosphorylated, was added to two further samples (untreated and treated with 1 μg/ml of PolyIC). The recombinant substrate yeast-His-eIF2α-wt was phosphorylated (Fig. 4, lanes 5–8) showing that PKR from Karpas299 can be activated by PolyIC. PKR was activated by 1 μg/ml PolyIC, but inhibited at the higher PolyIC concentration of 10 μg/ml. PKR is known to display a bell-shaped activation curve, whereby

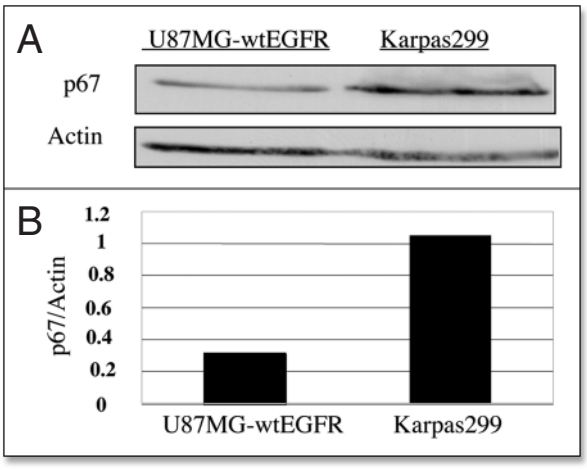

Figure 6. p67 level is higher in Karpas299 cells than in U87MG-wtEGFR cells. Karpas299 and U87MG-wtEGFR cells were lysed, electrophoresed and blotted. Probing the blot with anti-p67 and anti-actin antibodies revealed higher level of p67 protein in Karpas299 cells in comparison to U87MG-wtEGFR cells (A), as can be seen also by normalizing p67 levels to actin levels (B).

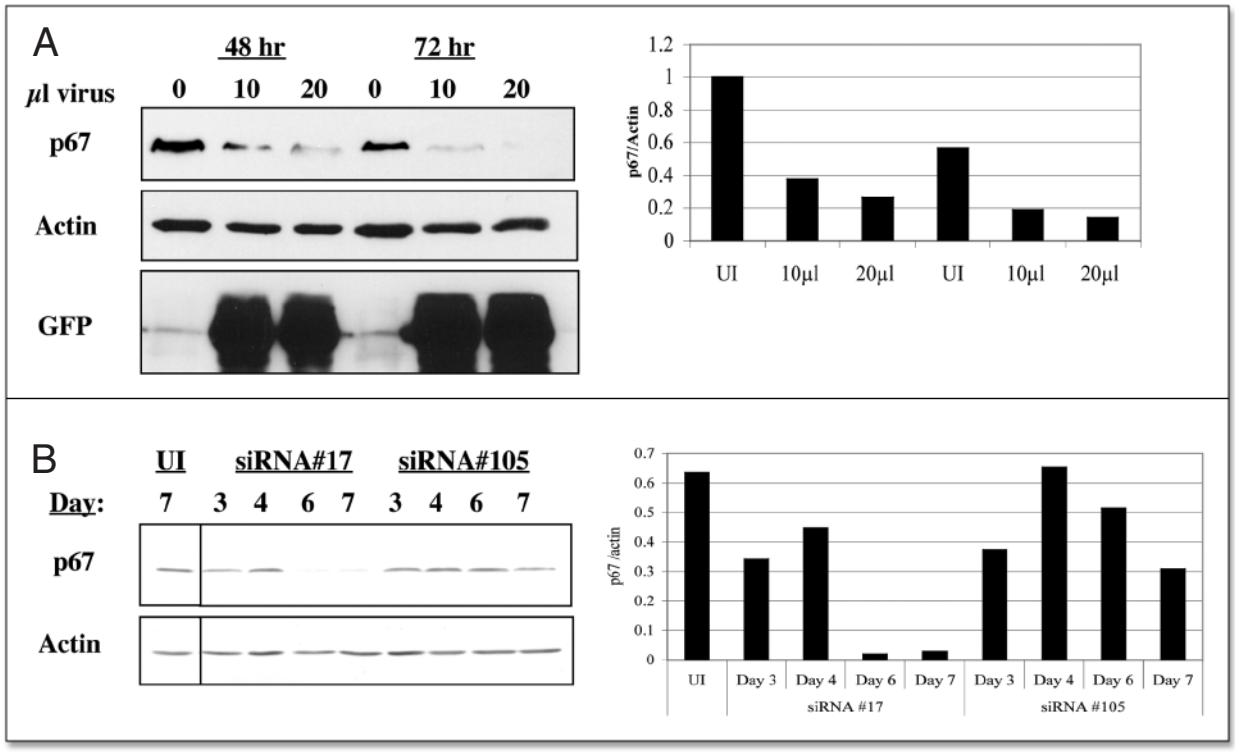

Figure 7. The effect of p67-As and p67-siRNAs encoding adenoviruses on p67 levels in Karpas299 cells. (A) Karpas299 cells were infected with p67-As encoding adenoviruses in two amounts (10 and 20 μl). Cells were harvested 48 and 72 hours after infection, lysed, electrophoresed and blotted. The blot was probed with anti-p67, anti-actin antibodies. The blot was also probed with anti-GFP antibody in order to validate the infection. As can be seen by the blot image, as well as by the graph that normalizes p67 levels to actin levels, infection of Karpas299 cells with p67-AS encoding viruses caused reduction in p67 levels. (B) Karpas299 cells were infected with p67-siRNA #17 or #105 encoding adenoviruses. Cells were harvested at four time points (three, four, six and seven days after infection), lysed, electrophoresed and blotted. The blot was probed with anti-p67 and anti-actin antibodies. As can be seen by the blot image, as well as by the graph that normalizes p67 levels to actin levels, infection of Karpas299 cells with these p67-siRNAs encoding viruses caused reduction in p67 levels, with a prominent effect of siRNA#17.

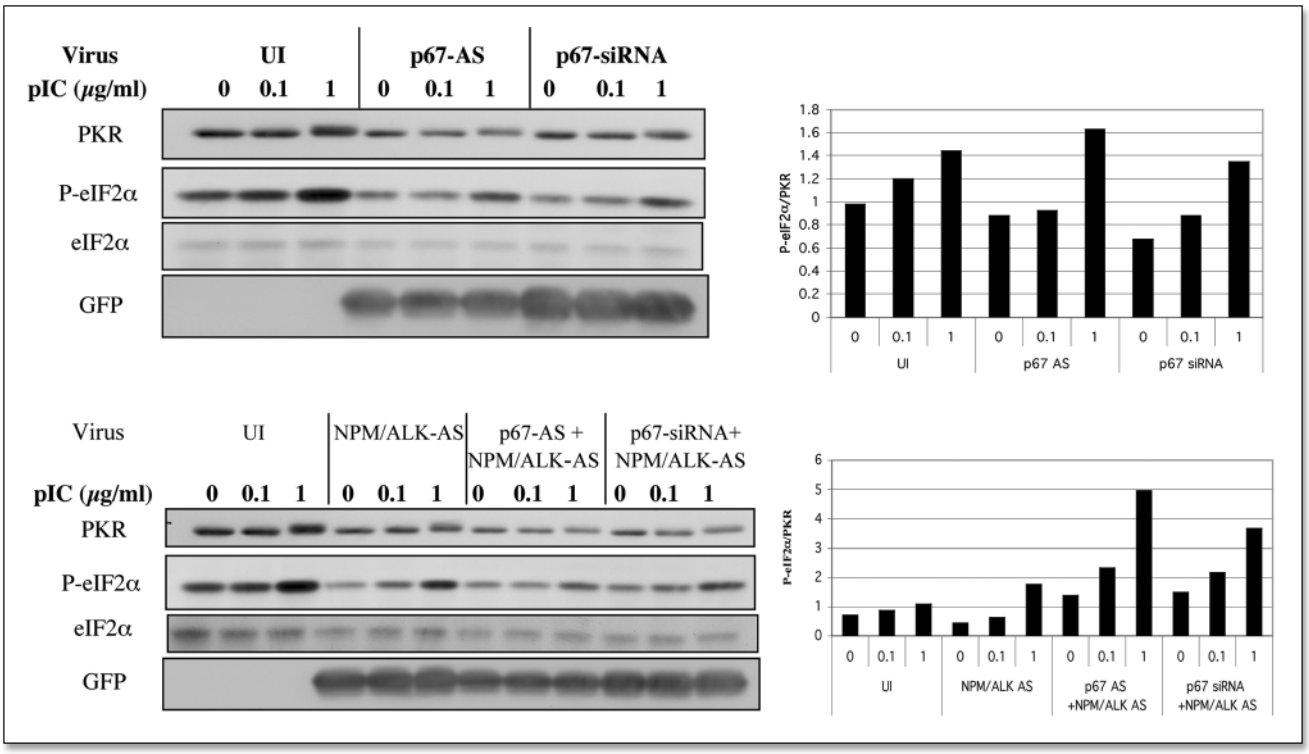

Figure 8. Reduction in p67 level elevated to some extent eIF2α phosphorylation. A cell-free nonradioactive PKR kinase assay was performed as described in Materials and Methods on lysates derived from cells infected adenoviruses encoding for NPM/ALK antisense, p67-antisense and p67-siRNA#17 (each one or in various combinations of coinfections). At the end of the reaction samples were electrophoresed and blotted. The blot was probed with anti-PKR, anti-P-eIF2α and anti-eIF2α antibodies. The blot was also probed with anti-GFP antibody in order to validate the infection. The graphs show normalization of P-eIF2α levels to PKR levels. As can be seen from the blot image, as well as by the graphs, the change in the phosphorylation state of eIF2α upon treatment with 1 μg/ml pIC in comparison to the basal phosphorylation or to the change achieved by treatment with 0.1 μg/ml pIC, is much higher in lysates derived from cell infected with p67-AS or p67-siRNA#17. This effect is even stronger for lysates derived from cell coinfected with p67-AS and NPM/ALK-AS.

high concentrations of dsRNA inhibit PKR activation.$_2$ As expected, PKR activation did not result in phosphorylation of the mutant recombinant substrate (Fig. 4, lanes 9–10). Though PKR did phosphorylate the recombinant wild type substrate, no change in the phosphorylation state of the endogenous eIF2α was achieved upon addition of PolyIC (Fig. 4, lanes 1–4).

In order to explore why the endogenous substrate was not phosphorylated in Karpas299 lysates, we looked for mutations in the sequence of eIF2α. The mRNA region flanking residue Ser51, which is subject to phosphorylation, was pulled out by RT-PCR and sequenced. The sequence obtained was identical to the published sequence (Accession #J02645) (data not shown).

Endogenous eIF2α is phosphorylated in response to stress-inducing agents. eIF2α phosphorylation can be induced by mechanisms other than PKR activation. Endoplasmic reticulum stress, for example, induces eIF2α phosphorylation through the activation of PERK.[12] Therefore, we examined whether the stress-causing agents, tunicamycin and sodium arsenate, induce eIF2α phosphorylation in Karpas299 cells. Arsenate treatment induced eIF2α phosphorylation dramatically within 30 minutes of treatment (Fig. 5). No phosphorylation was observed, however, 24 hours after arsenate treatment. This is probably due to the overall decrease in protein levels, as the low levels of PKR indicate. Tunicamycin also induced eIF2α phosphorylation, within 24 hours of treatment. Thus, phosphorylation of eIF2α can be induced in Karpas299 cells by stress-inducing agents. These data suggest that the ability of endogenous PKR to phosphorylate endogenous eIF2α is inhibited, but there is no defect in eIF2α itself. The ability of the endogenous PKR to phosphorylate exogenous eIF2α in response to PolyIC excluded the possibility that PKR is inhibited in Karpas299 cells, although a number of well-documented PKR inhibitors have been reported.[13-15]

p67 level is higher in Karpas299 cells than in U87MG-wtEGFR cells. We next considered the cellular glycoprotein, p67, which, in its glycosylated form, binds eIF2 and protects it from phosphorylation.[16-18] This inhibitor affects PKR activity without affecting its activation, making it a potential candidate for repression of PKR activity in Karpas299 cells. We therefore compared the level of p67 in Karpas299 cells with its level in U87MG-wtEGFR cells, which do die upon PKR activation.[4] Figure 6, shows that p67 levels are significantly higher in Karpas299 cells than in U87MG-wtEGFR cells.

Reducing p67 levels in Karpas299 cells and its consequence. In order to test the hypothesis that p67 prevents eIF2α phosphorylation in Karaps299 cells, we examined whether reducing p67 expression using anti-sense and RNAi technologies would alleviate the inhibition of eIF2α phosphorylation. Infection of Karpas299 cells with adenoviruses encoding p67-antisense reduced p67 levels within 48 hours of infection (Fig. 7A). This effect was even stronger 72 hours after infection. Infection of Karpas299 cells with adenoviruses encoding p67-siRNA#17 also reduced p67 levels, as seen six days after infection (Fig. 7B). Infection with adenoviruses encoding p67-siRNA #105 reduced p67 levels, but to a lesser extent than p67-siRNA#17.

Karpas299 cells were infected with adenoviruses encoding NPM/ALK antisense, p67-antisense and/or p67-siRNA#17, in various combinations, in order to achieve maximal reduction in p67 levels. We next compared eIF2α phosphorylation induced by PolyIC in these cells to cells in which p67 was not inhibited (Fig. 8). We observed enhanced phosphorylation in lysates obtained from cells with reduced levels of p67. Coinfection with adenoviruses encoding p67-antisense or RNAi plus adenoviruses encoding NPM/ALK antisense was most effective at inducing phosphorylation of eIF2α. These findings clearly demonstrate that when eIF2 is not protected by p67, activation of PKR by the NPM/ALK antisense can lead to eIF2α phosphorylation.

Reducing p67 levels does not affect cell growth. Infection of Karpas299 cells with adenoviruses encoding NPM/ALK antisense, p67-antisense and p67-siRNA#17 (separately or in various combinations) did not inhibit growth (data not shown). Thus, a decrease in p67 is insufficient to reconstitute the inhibitory effect of PKR. There are probably other, as yet undefined mechanisms by which Karpas299 cells resist PKR-induced growth inhibition.

CONCLUSIONS

The inhibition of cancer growth and progression is one of the major challenges facing modern medicine. Harnessing PKR for the selective killing of cancer cells, could serve as a powerful strategy for treating cancer and has been shown to be effective on an in vivo mouse model of glioblastoma.[4] Moreover, our finding that the prostate cancer cell line LNCaP is sensitive to PKR induced cell killing,[19] suggests that activation of PKR might be useful in treating some prostate cancers. However, applying the dsRNA killing approach to the T cell NHL line Karpas299, did not result in cell death. We have shown that eIF2α phosphorylation upon PKR activation is diminished in this cell line. Reduction of p67 by expression of specific antisense or RNAi increased to some extent the phosphorylation of eIF2α. Nevertheless, cell death was not achieved. These findings suggest that in the Karpas299 cell line strong anti-apoptotic mechanisms may preclude the utilization of the PKR killing strategy. Indeed, a growing body of evidence suggests that PKR activity is inhibited or impaired in various cancer cell lines, such as Ras-transformed cells,[20] leukemic cells from B cell chronic lymphocytic leukemia patients[21] and breast cancer cells.[22] Moreover, a B cell NHL line, expressing the Bcl2/IgH translocation, was also resistant to PKR activation (data not shown). This implies that the use of the PKR pathway to induce selective killing of cancer cells will only be successful in some cancers, implying the need to determine the status of the PKR pathway in a given cancer. A better understanding of the mechanisms that bypass PKR-induced cell death will eventually lead to development of appropriate strategies to achieved PKR-induced killing even in resistant tumors.

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